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(54) Title: IMPROVEMENTS IN OR RELATING TO CONTRAST AGENTS

(57) Abstract

Microparticulate materials which are capable of chemical generation of gas after formulation with an appropriate carrier liquid, e.g. water for injection, and/or after administration to a subject, e.g. as a result of exposure to blood or other body fluids, may be used as contrast agents in diagnostic imaging, particularly ultrasound and magnetic resonance imaging.

"Improvements in or relating to contrast agents"

This invention relates to novel contrast agents, more particularly to new microparticulate contrast agents of use in diagnostic imaging.

Ultrasound imaging is based on penetration of ultrasound waves, e.g. in the frequency range 1-10 MHz, into a human or animal subject via a transducer, the ultrasound waves interacting with interfaces of body tissues and fluids. Contrast in an ultrasound image derives from differential reflection/absorption of the sound waves at such interfaces; results may be enhanced by the use of Doppler techniques, including the use of colour Doppler to evaluate blood flow.

It has long been realised that it may be advantageous to increase the difference in acoustic properties of different tissues/fluids using contrast agents, and since the use of indocyanine green in 1968 as the first ultrasound contrast agent many other potential agents have been examined. These include emulsions, solid particles, water-soluble compounds, free gas bubbles and various types of encapsulated gas-containing systems. It is generally accepted that low density contrast agents which are easily compressible are particularly efficient in terms of the acoustic backscatter they generate; gas-containing and gas-generating systems thus tend to exhibit markedly greater efficacy than other types of contrast agent.

Three ultrasound contrast agents are now commercially available or in late clinical development, these being Echovist®, based on gas-containing galactose microcrystals; Levovist®, comprising gas-containing galactose microcrystals coated with fatty acid; and Albunex®, which comprises gas bubbles encapsulated by partially denatured human serum albumin.

Gas-containing contrast media are also known to be effective in magnetic resonance (MR) imaging, e.g. as susceptibility contrast agents which will act to reduce MR signal intensity. Oxygen-containing contrast media also represent potentially useful paramagnetic MR contrast agents.

Furthermore, in the field of X-ray imaging it has been observed that gases such as carbon dioxide may be used as negative oral contrast agents.

A general disadvantage of most existing gas-containing and gas-generating contrast agents is their relative lack of stability in vivo. This is a particular problem in areas such as echocardiography, where there is a need for improved contrast agents capable of generating microbubbles sufficiently small to pass through the pulmonary capillary bed (i.e. typically having a size of less than about 10 μm, preferably less than about 7 μm) and so permit visualisation of the left side of the heart, and which are preferably sufficiently stable to survive several passages of circulation.

It is also desirable that contrast agents should exhibit good storage stability over substantial periods of time, e.g. up to more than one year, preferably 2 to 3 years or more.

The present invention is based on our finding that microparticulate materials which are capable of chemical generation of gas after formulation with an appropriate carrier liquid, e.g. water for injection, and/or after administration to a subject, e.g. as a result of exposure to blood or another body fluid, may be useful as contrast agents in diagnostic imaging.

Such agents may be distinguished from existing microparticulate contrast agents such as the above-mentioned Echovist® and Levovist®, where microbubble generation is essentially only a physical process involving gas entrained on or in the microparticles, e.g. as inclusions in the voids of their crystal

structures and/or adhered to their surfaces. It will be appreciated that use of the contrast agents of the invention may lead to generation of microbubbles from such physically adsorbed gas as well as from chemically generated gas, and that this may enhance the overall intensity of the contrast effect.

Contrast agents containing chemically gas-generating substances encapsulated in liposomes have previously been described in, for example, WO-A-9109629. Since stable liposomes of necessity require a hydrophilic core, the encapsulated substances will typically be present as a solution in water or another relatively hydrophilic solvent; such contrast agents will be limited in their contrast effect by the maximum loading of gas-generating substance which may be introduced into solution in the liposome cores. Furthermore, such liposome products will tend to exhibit lower long term storage stability than dry microparticulate materials according to the present invention, as a result of factors such as vesicle fusion and leakage.

According to one aspect of the invention there is provided a contrast agent comprising a microparticulate substance capable of chemically generating gas upon formulation of the contrast agent in a carrier liquid and/or upon administration of the formulated contrast agent to a human or animal subject.

Such contrast agents may be used in a variety of diagnostic imaging techniques, including ultrasound, MR and X-ray imaging, their uses in diagnostic ultrasonic imaging and MR imaging, e.g. as susceptibility contrast agents, constituting preferred features of the invention.

The gas-generating substance may, for example, be a single compound which reacts chemically (which term is used herein to include enzymatically) to produce gas following administration to a subject, e.g. as a result

of decomposition induced thermally or by pH change or as a result of enzymatic degradation. Thus, for example, non-toxic inorganic and organic carbonates, e.g. alkali metal and alkaline earth carbonates and bicarbonates, arginine carbonate and compounds of formula RO.CO.OM where R is an organic group and M represents a physiologically acceptable cation, will generate carbon dioxide in the conditions of pH prevailing in the blood stream, as will compounds such as aminomalonates.

Carboxylic acids such as malonic acid, α-cyano acids, α-nitro acids, α-aryl acids, α-keto acids, α,α,α-trihalo acids, β-keto acids and β,γ-unsaturated acids decarboxylate relatively easily and may do so spontaneously <u>in vivo</u>, with generation of carbon dioxide.

Methylene diesters (e.g. prepared using techniques such as are described in WO-A-9317718 and WO-A-9318070, the contents of which are incorporated herein by reference) are cleaved by common esterases leading to evolution of carbon dioxide. Such double ester derivatives, e.g. of compounds such as dextrans, may therefore provide useful contrast agents in accordance with the invention.

Hydrogen peroxide, which may be present as an anti-oxidant-stabilised solid formulation or particle matrix, as a polyvinylpyrrolidone-hydrogen peroxide complex (see e.g. WO-A-9107184) or in precursor form, e.g. as sodium perborate tetrahydrate (see e.g. EP-A-0253772) or urea peroxide (see e.g. WO-A-9011248), is enzymatically degraded with evolution of oxygen.

It will be appreciated that the above contrast agents according to the invention remain stable after formulation, e.g. as solutions in injectable media such as water for injection, gas generation not commencing until actual administration. Such formulations constitute a further feature of the invention.

Another category of gas-generating substance

comprises compounds which react with water to generate gas; contrast agents using such compounds will begin to produce microbubbles immediately upon formulation into, e.g. water for injection. Representative compounds of this type include hydrides such as sodium borohydride or calcium hydride; acetylenides such as sodium acetylenide; carbides such as calcium carbide; N-carboxy anhydrides (see e.g. J. Am. Chem. Soc. 112 (1990), pp. 7414-7416), which react to yield carbon dioxide and an amino acid; and polycarbonates (see e.g. Pope et al. in Org. Synth. Coll. Vol. VI (1988), p. 418), e.g. compounds of formula

$$(CH_3)_3C.O.(CO_2)_n.C(CH_3)_3$$

where n is at least 2, which react with water to generate carbon dioxide.

Alternatively the gas-generating substance may comprise a plurality of compounds, which may be stored separately or in combination, and which interact when, for example, they are formulated into water for injection. Examples include traditional effervescent systems, typically containing a carbonate or bicarbonate (e.g. with a non-toxic alkali or alkaline earth metal) and an organic acid such as tartaric, succinic or citric acid. Other representative combined formulations include calcium percarbonate/sodium bicarbonate/citric acid, 5-nitrofuryl acrylate/ethylenediaminetetraacetic acid/ascorbic acid/tartaric acid/sodium metabisulphite/ sodium bicarbonate, and long-chain polyphosphates/ sodium bicarbonate.

The microparticulate material may if desired be stabilised, e.g. by being coated with or encapsulated in an appropriate biocompatible material, which may for example be chosen to be dissolvable and/or biodegradable. Representative materials thus include polyethylene glycols, pluronics, albumin, gelatin,

starch, collagen, dextrans, polylactide/polyglycolide, block copolymers and biodegradable polymers such as are described in WO-A-9204392, WO-A-9317718 and WO-A-9318070. The coating/encapsulation may incorporate ionophores such as nigericin to facilitate proton transfer therethrough in cases where the gas-generating substance is activated by pH change.

The microparticulate material may advantageously be stabilised in proliposome form, e.g. as described by Payne et al. in J. Pharm. Sci. 75 (1986), pp. 325-329, Katare et al. in J. Microencapsulation 7 (1990), pp. 455-462 and Ibid. 8 (1991), pp. 1-7, the contents of which are incorporated herein by reference. Essentially such products comprise microparticulate material coated with liposome generating material (e.g. a phospholipid such as phosphatidylcholine, hydrogenated phosphatidylcholine or hydrogenated phosphatidylserine) in dry form. Products of this type typically comprise dry, free-flowing powders and exhibit particularly good long term storage stability. Liposome formation will normally accompany gas generation when the product is formulated in an aqueous carrier liquid such as water for injection. Alternatively the coating material may be such that it is substantially impermeable or otherwise inert to the carrier liquid but is modified or activated on or immediately prior to administration, e.g. to exhibit enhanced permeability, for example as a result of pH change or enzyme activity, so leading to liposome formation and gas generation in vivo following administration. Aqueous suspensions and dispersions of this latter class of proliposomes may thus exhibit good storage stability and constitute a further feature of the invention. The stability of such aqueous formulations may if desired be enhanced by appropriate selection of conditions such as pH, for example by buffering the formulation to slight alkalinity, to ensure that substantially no gas generation occurs prior to their administration.

It will be appreciated that the liposomes generated by contrast agents in proliposome form may assist in stabilising the microbubbles which are generated by virtue of their long residence times in e.g. the vascular system.

If desired the microparticulate material in contrast agents according to the invention may incorporate a solute serving to generate an osmotic gradient to enhance diffusion of fluid through any coating into the material.

The stability of the microbubbles may in general be enhanced by the microparticles of the gas-generating substance themselves acting as condensation nuclei. The microparticles may also have porous or spongy structures, e.g. containing the gas-generating material in pores or networks of the structure or having gas pockets or cavities formed on the surface of the particles; the flexibility of such structures will enhance their echogenicity relative to more rigid gas-containing systems.

The contrast agents of the invention may be prepared by any convenient method, e.g. by micronising the gas-generating substance. Any desired coating or encapsulating material may be applied before or after such micronisation. Thus, for example, a preferably hydrophilic micronised gas-generating substance may be dispersed in a volatile lipophilic solvent in which the desired coating material is dissolved before, during or after the dispersion step, the solvent thereafter being removed, e.g. under reduced pressure, to yield a coated microparticulate product according to the invention.

In general conventional micronisation techniques such as grinding or milling may be employed; ball-milling may be particularly convenient.

Coating/encapsulation may likewise be effected using conventional methods, e.g. fluidised bed, spray, moulding, dipping, coacervation-phase separation,

multiorifice centrifugal and solvent evaporation techniques, to give coatings having appropriate composition, thickness and permeability, in one or more layers.

The contrast agents of the invention may, for example, be administered enterally or parenterally, although there may be advantages in particular applications in administration directly into body cavities such as the Fallopian tubes. In general, however, intravascular administration, most commonly by intravenous injection, is most likely to be employed, in order to enhance vascular imaging, including cardiac and extracardiac perfusion.

It will be appreciated that contrast agents for intravenous administration should generate microbubbles small enough to pass through the capillary bed of the pulmonary system. The agents should therefore preferably be such as to generate microbubbles having diameters of less than 10 μm, preferably in the range 0.2-8 μm, e.g. 0.3-7 μm; the microparticles may, for example, conveniently have an average size of 1-7 μm, e.g. 1-4 μm. Substantially larger microparticle and microbubble sizes, e.g. up to 500 μm, may be useful in applications such as gastrointestinal imaging.

The following non-limitative examples serve to illustrate the invention.

Example 1

Phosphatidylcholine (2.5g) was added to a suspension of anhydrous sodium bicarbonate (10g) in chloroform (30 ml) and allowed to dissolve. The solvent was then removed under reduced pressure at 40°C to yield a solid product.

A sample of this product (100 mg) was dissolved in aqueous glucose (5 ml of a 50 mg/ml solution), and the solution was acidified to pH 2 through addition of hydrochloric acid and heated to 50°C. A turbid suspension was formed in which the particles present tended to float to the top. Light microscopy showed that liposomes were formed and that the vesicles contained gas.

Example 2

A suspension of fine-grained anhydrous sodium carbonate (1.6g) in chloroform (20 ml) was sonicated for several minutes to break up agglomerates. Hydrogenated phosphatidylcholine (280 mg) was added and dissolved in the suspension with agitation. The solvent was then removed under reduced pressure at 40°C to yield a solid powder.

A sample of this product (100 mg) was added to aqueous ascorbic acid (2 ml of a 25 mg/ml solution), leading to formation of a turbid suspension in which the particles present tended to float to the top. Light microscopy showed that liposomes were formed and that the vesicles contained gas.

Example 3

A suspension of fine-grained anhydrous sodium carbonate (1.6g) in chloroform (20 ml) was sonicated for several minutes to break up agglomerates. Hydrogenated

phosphatidylserine (280 mg) was added and dissolved in the suspension with agitation and glycerol (4 ml) was added. The solvent was then removed under reduced pressure at 40°C to yield an anhydrous concentrate.

A sample of this product (200 mg) was added to aqueous glucose (4 ml of a 50 mg/ml solution), whereupon gas formation by the sodium carbonate was induced by protons from the serine moiety of the phospholipid, leading to formation of a turbid suspension in which the particles present tended to float to the top. Light microscopy showed that liposomes were formed and that the vesicles contained gas.

Example 4

The acoustic effect of the formulated products from Examples 1-3 was determined by further diluting them ten-fold with Isoton II (Coulter Electronics Limited, Luton, England), placing the diluted samples in cells in a water bath maintained at 37°C and measuring the acoustic backscatter using a 3.5 MHz single element transducer in a pulse-reflection technique. In all cases a strong acoustic backscatter from the interior of the cell was observed, whereas a reference measurement on a cell containing only Isoton II showed no acoustic backscatter.

Example 5

A fine-grained powder of sodium carbonate and sodium bicarbonate (1:1 w/w, 1.36g) was dispersed in hexane (20 ml) containing Aerosol OT (1.75g). Tween 60 (1.0g) dissolved in water (50 ml) was added and the resulting mixture was emulsified using an Ystral homogeniser, yielding a fine emulsion.

A sample of this product (2 ml) was injected into phosphate buffer (5 ml). The resulting mixture was

observed to exhibit an echogenic effect in vitro, the signal being stable for 20 minutes.

Claims

1. A contrast agent comprising a microparticulate substance capable of chemically generating gas upon formulation of the contrast agent and/or upon administration of the formulated contrast agent to a human or animal subject.

2. A contrast agent as claimed in claim 1 wherein the substance capable of chemically generating gas is selected from alkali metal and alkaline earth metal carbonates and bicarbonates, arginine carbonate, compounds of formula RO.CO.OM where R represents an organic group and M represents a physiologically acceptable cation, carboxylic acids which spontaneously decarboxylate in vivo, enzymically degradable methylene diesters, solid formulations of or precursors for hydrogen peroxide, hydrides, acetylenides, carbides, N-carboxy anhydrides, polycarbonates, and effervescent formulations comprising at least one carbonate or bicarbonate and at least one organic acid.

3. A contrast agent as claimed in claim 2 wherein the substance capable of chemically generating gas is selected from sodium carbonate, sodium bicarbonate and mixtures thereof.

4. A contrast agent as claimed in any of the preceding claims wherein the microparticulate substance is coated with or encapsulated in a biocompatible material.

5. A contrast agent as claimed in claim 4 wherein the microparticulate substance is coated with a liposome generating material in dry form.

6. A contrast agent as claimed in claim 5 wherein the liposome generating material is selected from

phosphatidylcholine, hydrogenated phosphatidylcholine and hydrogenated phosphatidylserine.

7. A contrast agent as claimed in claim 5 or claim 6 wherein the liposome generating material is unreactive when stored in an aqueous formulating medium but is capable of promoting liposome generation in vivo following administration of the contrast agent or following adjustment of the formulation prior to administration.

8. Non-aqueous suspensions and concentrates containing a contrast agent as claimed in any of the preceding claims.

9. Formulated forms of a contrast agent as claimed in any of the preceding claims comprising a substance capable of chemically generating gas which is activated only upon administration.

10. A formulated form as claimed in claim 9 comprising an aqueous suspension or dispersion of a contrast agent as claimed in claim 7.

11. A process for the preparation of a contrast agent as claimed in claim 1 which comprises micronising the substance capable of chemically generating gas, and/or applying a coating or encapsulating material to such a substance before or after such micronisation.

12. Use of a contrast agent as claimed in any of claims 1 to 10 in diagnostic imaging.

13. Use of a contrast agent as claimed in any of claims 1 to 10 in ultrasound imaging.

14. Use of a contrast agent as claimed in any of claims 1 to 10 in magnetic resonance imaging.

15. A method of generating enhanced images of a human or non-human animal body which comprises administering to said body a contrast agent as claimed in any of claims 1 to 10 and generating an ultrasound or magnetic resonance image of at least a part of said body.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 94/00522

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,4 900 540 (P. J. RYAN) 13 February 1990 see column 2, line 52 - line 68; claims; example 2 ---	1-15
P,X	WO,A,93 17718 (NYCOMED IMAGING A/S) 16 September 1993 cited in the application see page 4, line 36 - line 12 see page 5, line 32 - page 6, line 2 see page 18, line 23 - line 34; claims 1,15-17 --- -/--	1-4,8-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 July 1994	04.08.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Berte, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 94/00522

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 117, no. 23, 7 December 1992, Columbus, Ohio, US; abstract no. 229280y, see abstract & PHOTOCHEM. PHOTOBIOL., vol.56, no.4, 1992 pages 441 - 445 WALTER C. EISENBERG ET AL. 'OXIDATION OF PHOSPHATIDYLCHOLINE MEMBRANES BY SINGLET OXYGEN GENERATED IN THE GAS PHASE' ---	1,2, 5-10, 12-15
X	US,A,5 147 631 (JOSEPH L. GLAJCH) 15 September 1992 see claims ---	1-4,8-15
X	WO,A,90 03800 (OTSUKA PHARMACEUTICAL CO.) 19 April 1990 -----	1-3,9, 11,12,14

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB94/00522

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 15 is directed to a method of treatment of (diagnostic method practised on) the human/animal body the saerch has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir ational Application No
PCT/GB 94/00522

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4900540	13-02-90	NONE		
WO-A-9317718	16-09-93	WO-A-	9318070	16-09-93
US-A-5147631	15-09-92	AU-A-	2002892	21-12-92
		EP-A-	0583401	23-02-94
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		CA-A-	2000112	04-04-90
		CH-A-	677880	15-07-91
		DE-D-	68909221	21-10-93
		DE-T-	68909221	03-03-94
		EP-A,B	0401377	12-12-90
		GB-A,B	2232592	19-12-90
		US-A-	5174987	29-12-92
		AU-B-	624231	04-06-92
		AU-A-	5254090	11-04-91